Synthesis and Biological Evaluation of 5-Fluoro-2′-deoxyuridine Phosphoramidate Analogs

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A series of alkylating phosphoramidate analogs of 5-fluoro-2′-deoxyuridine has been prepared and their growth inhibitory activity evaluated against murine L1210 leukemia and B16 melanoma cells in vitro. These compounds were designed to undergo intracellular release of the phosphoramidate anions, which it was hoped would function as irreversible inhibitors of thymidylate synthase. The expectation was that binding of the nucleoside moiety would be followed by alkylation of the enzyme via the phosphoramidate. The chloride, bromide, iodide, and tosylate analogs were highly potent inhibitors of L1210 cell proliferation, with increased inhibition observed at both higher drug concentrations and longer exposure times. Addition of thymidine completely reversed the inhibition for all compounds, suggesting that these compounds are acting via inhibition of thymidylate synthase. Although the nonalkylating morpholine analog **1f** was ca. 50-fold less potent than the methyl(chloroethyl)amino compound, the piperidine analog **1g** was only 2-fold less potent, confirming that nitrogen basicity may be as important as the presence of an alkylating group. Addition of thymidine reversed the growth inhibition of the morpholine and piperidine analogs, suggesting that these compounds may also undergo intracellular conversion to 5-fluoro-2′-deoxyuridine 5′monophosphate. The thymidine and deoxyuridine derivatives **2** and **3** showed minimal growth inhibition in the L1210 assay. The alkylating analogs showed modest cytotoxicity against B16 melanoma cells, and the potency of the analogs was more dependent upon the alkylating moiety than on the 5-substituent.

Introduction

5-Fluoro-2′-deoxyuridine 5′-monophosphate (F-dUMP), a major metabolite of the antitumor agent 5-fluorouracil (5-FU), is a potent inhibitor of thymidylate synthase.[1,2] Despite the potency of 5-FU, however, low response rates and the development of resistance represent major problems in the clinical application of this drug.[3] Although direct administration of the nucleotide might circumvent these problems, nucleotides penetrate cells poorly and are readily dephosphorylated extracellularly. The development of neutral nucleotide prodrugs that can cross the cell membrane and liberate nucleotide intracellularly is an area of intense investigation.[4–7] On the basis of our interest in strategies to control the reactivity of phosphoramidate alkylating agents, we have designed and synthesized a series of incipient alkylating F-dUMP prodrugs, **1** (e.g., **A**, Scheme 1). The perhydrooxazine ring was introduced in order to deliver the 5-fluoro-2′-deoxyuridine 5′-phosphoramidates (e.g., **B**, Scheme 1) intracellularly via enamine expulsion or hydrolysis and elimination from the resulting aldehyde.[8] It was anticipated that the F-dUMP analogs **B** might have reasonable affinity for thymidylate synthase and that formation of the electrophilic aziridinium phosphoramidate would provide a route for irreversible alkylation of this enzyme at the active site (**C**, Scheme 1). Alternatively, the aziridinium phosphoramidate may undergo P–N bond cleavage to generate F-dUMP (**D**) directly. We report here the synthesis and growth inhibitory activity of these compounds and provide mechanistic evidence to suggest that release of F-dUMP rather than enzyme alkylation accounts for the growth inhibitory effects.

Results and Discussion

Chemistry. The synthesis of the target compounds was carried out as shown in Scheme 2. The 3′-protected nucleosides **7**, **10**, and **13** were prepared by treating the 2′-deoxynucleosides with triphenylmethyl chloride and *tert*-butyldimethylsilyl chloride followed by detritylation. The phosphorylating agents **6** were prepared by reaction of $POCl_3$ with the appropriate amine salt and triethylamine followed by treatment with the lithium salt of 4-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane (**5**). The 3′-protected nucleosides were then converted to the alkoxide and phosphorylated with **5** to give **8**, **11**, and **14**. The acetonides were converted to the perhydrooxazines **9**, **12**, and **15** via a three-step sequence involving hydrolysis of the acetonide, cleavage of the diol with sodium periodate, and trapping of the resulting aldehyde with 4-methyl-4-amino-2-pentanol. Finally, the 3′-protecting group was removed to give the 2′-deoxynucleoside phosphoramidates **1–3**. For the bis-(2-bromoethyl) compounds **1e**, **2e**, **and 3e**, both the acetonide and *tert*-butyldimethylsilyl protecting groups were cleaved prior to the periodate reaction, so that generation and trapping of the aldehyde afforded **1e**, **2e**, and **3e** directly. Although the products **1–3** were obtained as a mixture of diastereomers, they were not further purified because all of the prodrug diastereomers produce the identical phosphoramidate enantiomer following activation. ^{31}P NMR experiments demonstrated that activation rates of the respective diastereomers were essentially identical under hydrolysis conditions.

Biological Activity. Compounds **1a–e** were evaluated for growth inhibitory activity against L1210 leukemia cells. The bromo analog **1a** is a potent inhibitor of cell proliferation using a 2-h drug treatment (Figure

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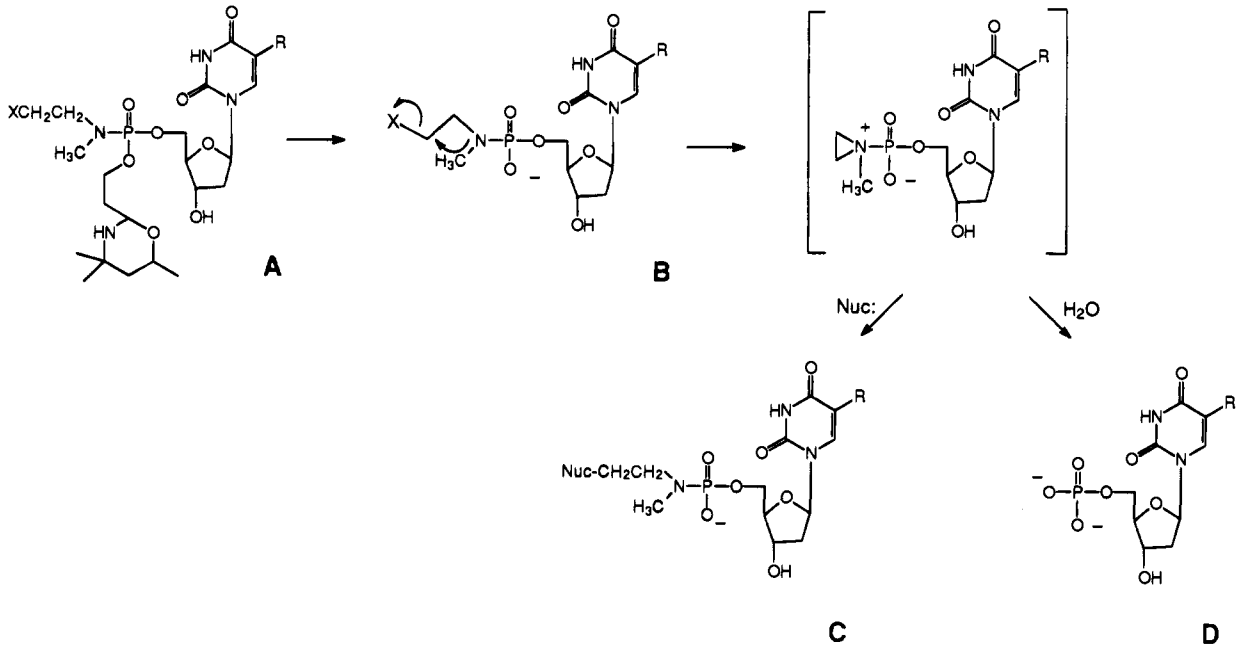
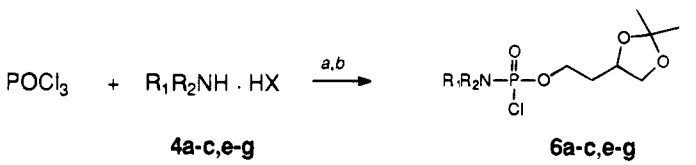
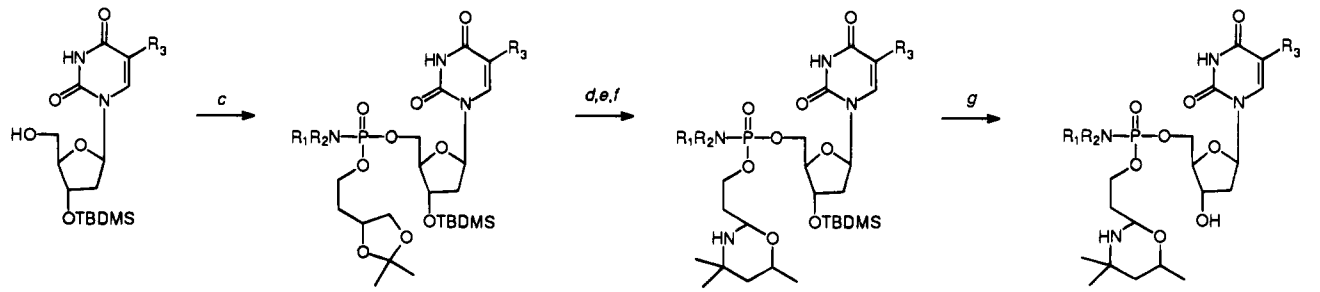
Scheme 1



Scheme 2[a]





7; R_3 = F
10; R_3 = H
13; R_3 = CH_3

8a-g; R_3 = F
11a,b,e; R_3 = H
14a,e; R_3 = CH_3

9a-d,f,g; R_3 = F
12a,b; R_3 = H
15a; R_3 = CH_3

1a-g; R_3 = F
2a,b,e; R_3 = H
3a,e; R_3 = CH_3

	R_1	R_2
a	CH_3	CH_2CH_2Br
b	CH_3	CH_2CH_2Cl
c	CH_3	CH_2CH_2I
d	CH_3	CH_2CH_2OTs

	R_1	R_2
e	CH_2CH_2Br	CH_2CH_2Br
f	$CH_2CH_2OCH_2CH_2$	
g	$CH_2CH_2CH_2CH_2CH_2$	

[a] (a) Triethylamine, CH_2Cl_2; (b) **5**, BuLi, THF, −78 °C; (c) LDA, THF, then **6**; (d) 80% AcOH, reflux; (e) $NaIO_4$, acetate buffer, pH 5.0, THF; (f) 4-methyl-4-amino-2-pentanol, CH_2Cl_2; (g) Bu_4NF, THF, 0 °C.

1). The inhibitory activity is completely blocked by addition of 5 μM thymidine, confirming that this compound is acting via inhibition of thymidylate synthase. The potency of **1a** increased with longer treatment times and was 1−2 orders of magnitude more potent than 5-FU using treatment times of 2, 8, 24, and 48 h (Figure 2 and Table 1). Surprisingly, the extent of inhibition was comparable for the bromo, iodo, and toluenesulfonyl analogs. This result is inconsistent with inhibition occurring via binding of the phosphoramidate anion to the active site because the spatial requirements of the tosyl group should preclude active site binding. The chloro analog **1b**, whose phosphoramidate would be expected to have a slower rate of aziridinium ion formation,[9] was less potent than **1a**,**c**,**d** at shorter exposure times but equipotent for the 24- and 48-h

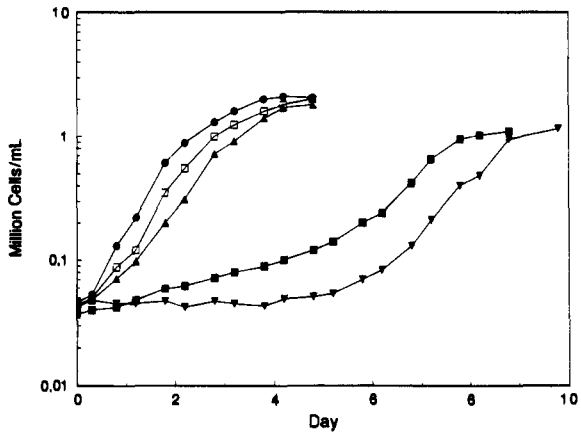


Figure 1. Growth inhibitory activity of **1a**. L1210 cells were treated with **1a** for 2 h in serum-free medium. The drug-containing medium was removed, the cells were washed, and complete medium was added. Aliquots were removed, and viable cells were counted at intervals for 10 d. In one experiment, complete medium also contained 5 μM thymidine. Concentration of **1a**: (●) 0 (control), (▲), 0.3 μM, (■) 1.0 μM, (▼) 3.0 μM, (□) 1.0 μM + 5 μM thymidine. Data represent the average of three experiments.

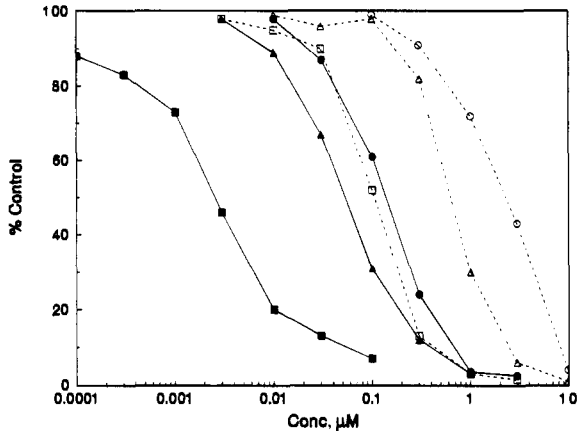


Figure 2. Dose response of **1a** (—) and 5-FU (···). L1210 cells were treated with drug for 2, 8, and 48 h. The drug-containing medium was removed, the cells were washed, complete medium was added, and the cells were incubated for 48 h total. Cell counts were obtained 48 h after the start of drug treatment. Treatment time: (●, ○) 2 h, (▲, △) 8 h, (■, □) 48 h. Data represent the average of three experiments.

Table 1. Growth Inhibition of Nucleoside Phosphoramidates against L1210 Cells in Vitro

				IC_{50}, μM			
				treatment time, h			
compd	R_1	R_2	R_3	2	8	24	48
1a	CH_2CH_2Br	CH_3	F	0.12	0.05	0.018	0.0025
1b	CH_2CH_2Cl	CH_3	F	0.3	0.16	0.025	0.0035
1c	CH_2CH_2I	CH_3	F	0.19	0.075	0.006	0.002
1d	CH_2CH_2OTs	CH_3	F	0.16	0.060	0.019	0.0025
1f	$-CH_2CH_2OCH_2CH_2-$		F	15	5	0.9	0.15
1g	$-CH_2CH_2CH_2CH_2CH_2-$		F	0.75	0.26	0.0240	0.008
2a	CH_2CH_2Br	CH_3	H	20	8.2	1.3	0.43
2b	CH_2CH_2Cl	CH_3	H	40	16.5	6	0.55
3a	CH_2CH_2Br	CH_3	CH_3	13	10.5	7	0.59
5-fluorouracil				2.2	0.63	0.22	0.12

exposure times. The nonalkylating morpholine and piperidine analogs also inhibited cell proliferation in a concentration- and time-dependent manner, and inhibition was reversed by thymidine for both analogs. Remarkably, the piperidine analog was only 2-fold less potent than the chloroethyl analog and 20-fold more potent than the morpholine compound. These data are consistent with a mechanism involving intracellular release of the phosphoramidate anion, aziridinium ion formation from the alkylating analogs and protonation of the nonalkylating analogs, and hydrolytic cleavage of the phosphoramidate zwitterions to generate F-dUMP. Presumably the piperidine analog is more potent than the morpholine analog because of the greater nitrogen basicity of the former, leading to more facile protonation and P–N bond cleavage. The deoxyuridine and thymidine derivatives **2a,b** and **3a** possess weak inhibitory activity, approximately 2 orders of magnitude less than their 5-fluoro counterparts. This activity cannot be explained by nucleotide release because the activation mechanism proposed would generate the nontoxic nucleotides dUMP and TMP, respectively. Although alkylation of some other cellular target by the aziridinium species may occur (**C**, Scheme 1), expulsion of *N*-methylaziridine is concomitant with cleavage to nucleotide, and this may account for the limited toxicity of these nonfluorinated compounds.

Table 2. Cytotoxicity of Nucleoside Phosphoramidates Against B16 Melanoma Cells in Vitro

compd	R_1	R_2	R_3	LC_{99}, μM
1a	CH_2CH_2Br	CH_3	F	101
1b	CH_2CH_2Cl	CH_3	F	375
1c	CH_2CH_2I	CH_3	F	95
1d	CH_2CH_2OTs	CH_3	F	141
2a	CH_2CH_2Br	CH_3	H	319
3a	CH_2CH_2Br	CH_3	CH_3	165
1e	CH_2CH_2Br	CH_2CH_2Br	F	100
2e	CH_2CH_2Br	CH_2CH_2Br	H	155
3e	CH_2CH_2Br	CH_2CH_2Br	CH_3	120
1g	$-CH_2CH_2CH_2CH_2CH_2-$		F	650
5-fluorouracil				>2000
4-hydroperoxycyclophosphamide				12

It is possible that these compounds are undergoing activation via a process involving extracellular generation of F-dUMP by the mechanism proposed above and that F-dUMP is converted to FUdR in the serum-containing medium. FUdR would then be taken up by the cells and converted to F-dUMP by thymidine kinase. To address this question, L1210 cells were treated with **1a** for 2 h in serum-containing and serum-free media. Growth inhibition was essentially identical in the presence and absence of serum, suggesting that serum phosphatases are not contributing significantly to the toxicity of these compounds.

The toxicity of several analogs against B16 cells was also evaluated in a clonogenic assay. 5-Fluorouracil was essentially nontoxic in this assay. The nonalkylating prodrug **1g** exhibited low cytotoxicity, suggesting either that F-dUMP is not an effective growth inhibitor in this cell line or that cleavage of the phosphoramidate anion to F-dUMP is impaired. The alkylating analogs showed modest cytotoxicity against B16 cells, approximately 1 order of magnitude less potent than 4-hydroperoxycyclophosphamide. This may result from the greater extent of P–N bond cleavage that occurs in the nucleotide analogs compared to phosphoramide mustard. It is interesting to note that the bifunctional analogs **1e**, **2e**, and **3e** are comparable in cytotoxicity to the potential monoalkylators.

NMR Studies. Confirmation of P–N bond cleavage in the activation of these compounds was sought using ^{31}P NMR. A solution of the deoxyuridine derivative **2a**

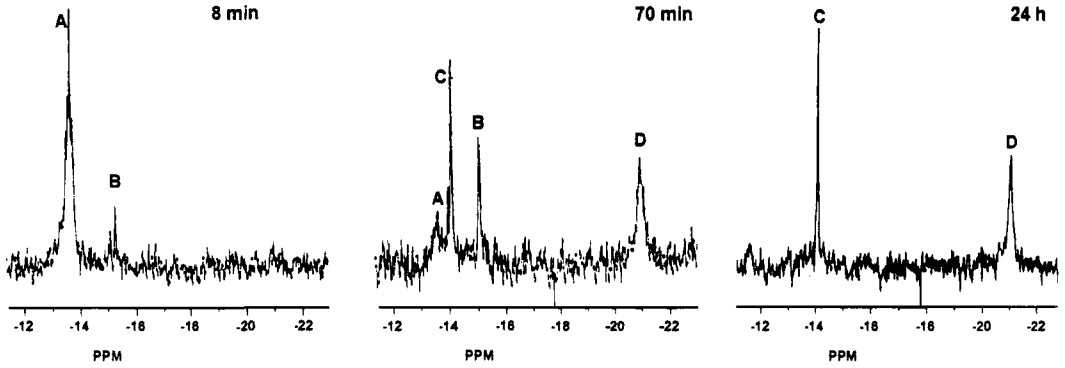


Figure 3. ^{31}P NMR spectra of **2a** (peak A) in 0.4 M cacodylate buffer, pH 7.4, 37 °C. See Scheme 1 for assignment of the resonances.

was prepared (20 mM in 0.4 M cacodylate buffer, pH 7.4, 37 °C), and the reactions were monitored by ^{31}P NMR (Figure 3). The resonance for **2a** (A, −13.7 ppm) was initially replaced by the resonance for the phosphoramidate anion (B, −15.1 ppm). The phosphoramidate anion resonance gradually disappeared and gave rise to two products: the hydroxyethyl product arising from attack of water on the aziridinium ion (C, −14.0 ppm) and the monophosphate arising from P−N bond cleavage (D, −21.0 ppm). The concomitant appearance of peaks C and D and the failure to observe conversion of peak C to D suggest that the monophosphate was generated via P−N bond cleavage of the aziridinium ion rather than cleavage of the (bromoethyl) or (hydroxyethyl)phosphoramidate. This was confirmed by repeating the experiment in the presence of sodium dimethyldithiocarbamate (1.2 equiv); this potent nucleophile will not react with the (bromoethyl)phosphoramidate but reacts rapidly with the aziridinium intermediate.[9] Again, the resonance for **2a** was replaced by the phosphoramidate resonance. However, the phosphoramidate was converted exclusively to the dithiocarbamate ester arising from aziridinium ion trapping (−14.5 ppm); there was no evidence for monophosphate formation under these conditions. The only mechanism by which dimethyldithiocarbamate can inhibit monophosphate formation in this experiment is by interception of a common intermediate, providing further support for P−N bond cleavage of the aziridinium ion intermediate as the mechanism for monophosphate formation.

Similar ^{31}P NMR experiments were carried out (20 mM in 0.4 M cacodylate buffer, pH 7.4, 37 °C) for the morpholine and piperidine analogs **2f,g**. In each case the resonance for the starting diester (−15.7 and −14.3 ppm, respectively) was replaced by the phosphoramidate anion resonance (−16.8 and −14.9 ppm, respectively) with a half-life of approximately 25 min. Surprisingly, however, the phosphoramidate anions were stable under these conditions for >12 h; P−N bond cleavage in aqueous buffer to produce the monophosphate was not observed in either case. Thus **1f,g** presumably undergo enzymatic activation, perhaps via a phosphoramidase. Although the potency of these compounds is most consistent with direct intracellular conversion to F-dUMP, more extensive catabolism cannot be ruled out.

During the course of this work, the crystal structure of thymidylate synthase with bound F-dUMP and cofactor was reported.[10] Examination of the active site indicates that the phosphate binding region is unlikely to accommodate the alkylating moieties contained in the phosphoramidates of these compounds. This suggests that the 5-fluoro analogs function as F-dUMP prodrugs and that any contribution of the alkylating moiety to growth inhibition must be occurring at another site.

Summary

The synthesis of a series of nucleoside phosphoramidate prodrugs has been achieved, and the fluorodeoxyuridine analogs demonstrate potent inhibition of L1210 cell proliferation that is reversed by thymidine. Although it was hoped that these compounds might exhibit a novel mechanism of irreversible thymidylate synthase inhibition, the data are consistent with these compounds acting as prodrugs for the intracellular delivery of F-dUMP.

The alkylating analogs may have a dual mechanism of action, whereby the intracellular activation process results in the release of an alkylating moiety and a nucleotide analog. Toxicity to cell lines that are exquisitely sensitive to thymidylate synthase inhibition results primarily from F-dUMP delivery, whereas the delivery of an alkylating agent may be the primary toxic event in other cell lines. The generality of this approach to nucleotide delivery is currently being explored.

Experimental Section

General Introduction. NMR spectra were recorded on a Bruker WP-270SY spectrometer. ^1H NMR spectra were measured at 270.19 MHz and are reported in the following format: chemical shift (multiplicity, number of protons). ^{31}P NMR spectra were measured at 109.37 MHz using a 10-mm VSP multinuclear probe with broad-band ^1H decoupling and are reported as ppm from triphenylphosphine oxide in toluene-d_8 present as a coaxial reference (triphenylphosphine oxide/toluene-d_8 has a chemical shift of 24.7 ppm relative to 85% phosphoric acid). Melting points were measured on a Meltemp apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and Midwest Microlab, Indianapolis, IN. Chromatographic separations were performed by flash chromatography on silica gel grade 60. All reactions were carried out under a nitrogen atmosphere unless otherwise specified, or reagents containing water were used. All organic solvents were distilled prior to use unless otherwise specified.

***N*-Methyl-*N*-(2-bromoethyl)amine hydrobromide (4a):** prepared as described previously[11] on a 133-mmol scale and isolated as a white solid (20.36 g, 70%); mp 66−74 °C; ^1H NMR ($CDCl_3$) δ 3.83 (t, 2H), 3.47 (t, 2H), 2.82 (s, 3H).

***N*-Methyl-*N*-(2-chloroethyl)amine Hydrochloride (4b).** Hydrogen chloride was bubbled into a stirred solution of 2-(methylamino)ethanol (10.00 g, 133-mmol) in CH_2Cl_2 (25 mL) until the mixture turned wet litmus paper red. The mixture was cooled to 0 °C, and thionyl chloride (15.82 g, 133-mmol) was added dropwise. The mixture was allowed to stir overnight at room temperature. The solvent was removed under

reduced pressure to give a white solid (16.60 g, 96%): mp 95–100 °C; ^{1}H NMR (DMSO-d_6) δ 4.00 (t, 2H), 3.36 (t, 2H), 2.81 (s, 3H).

N-Methyl-N-(2-iodoethyl)amine hydroiodide (4c): prepared as described for **4a** on a 133-mmol scale and isolated as an off-white solid (38.73 g, 93%); mp 134–136 °C; ^{1}H NMR ($CDCl_3$) δ 3.32 (m, 4H), 2.60 (s, 3H).

4-(2-Hydroxyethyl)-2,2-dimethyl-1,3-dioxolane (5). 1,2,4-Butanetriol (5.0 g, 47-mmol) was dissolved in MeOH (5 mL). Acetone (20 mL), $MgSO_4$ (ca. 500 mg), and *p*-TsOH (25 mg) were added, and the mixture was allowed to stir for 5 days at room temperature. The mixture was filtered and the filtrate concentrated under reduced pressure. CH_2Cl_2 (20 mL) and water (20 mL) were added, the layers were separated, and the organic layer was washed with water (2 × 20 mL). The organic layer was dried ($MgSO_4$) filtered and the filtrate concentrated under reduced pressure to afford **5** as a clear oil (5.15 g, 75%): ^{1}H NMR ($CDCl_3$) δ 4.23 (dd, 1H), 4.04 (dd, 1H), 3.72 (m, 2H), 3.54 (dd, 1H), 2.55 (br s, 1H), 1.77 (dt, 2H), 1.37 (s, 3H), 1.31 (s, 3H).

3′-O-(*tert*-Butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (7). To a solution of 5-fluoro-2′-deoxyuridine (1.00 g, 4.06 mmol) in pyridine (7 mL) were added triphenylmethyl chloride (1.25 g, 4.47 mmol) and 4-(dimethylamino)pyridine (0.4 g, 3.05 mmol). The mixture was allowed to stir at 80 °C for 30 min. The pyridine was removed under reduced pressure and the residue dissolved in DMF (4 mL). Imidazole (0.67 g, 9.77 mmol) and *tert*-butyldimethylsilyl chloride (0.74 g, 4.88 mmol) were added, and the mixture was allowed to stir overnight at room temperature. The DMF was removed under reduced pressure, and the residue was dissolved in 80% aqueous acetic acid (5 mL). The mixture was allowed to reflux for 30 min, cooled, and neutralized with NaOH. The mixture was lyophilized, and the residue was purified by chromatography (1:1 EtOAc:hexanes) to give **7** as a white solid (709 mg, 48%): mp 138–142 °C; R_f 0.65 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 1H), 6.23 (dd, 1H), 4.49 (dt, 1H), 3.98 (m, 2H), 3.84 (m, 1H), 2.26 (m, 2H), 0.90 (s, 9H), 0.10 (s, 6H).

3′-O-(*tert*-Butyldimethylsilyl)-2′-deoxyuridine (10): prepared as described for **7** using 2′-deoxyuridine on a 22-mmol scale. The residue was purified by chromatography (1:4 EtOAc:hexanes) to give **10** (3.13 g, 41%) as a white solid: mp 160–170 °C; R_f 0.65 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.62 (d, 1H), 6.16 (dd, 1H), 5.73 (d, 1H), 4.49 (m, 1H), 3.93 (m, 2H), 3.76 (m, 1H), 2.30 (m, 1H), 2.19 (m, 1H), 0.89 (s, 9H), 0.09 (s, 6H); FAB MS *m*/*z* 343 (M + H$^+$).

3′-O-(*tert*-Butyldimethylsilyl)thymidine (13): prepared as described for **7** using thymidine on a 41-mmol scale. The residue was purified by chromatography (1:4 EtOAc:hexanes) to give **13** (10.35 g, 71%) as a white solid: mp 90 °C; R_f 0.69 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.62 (d, 1H), 6.13 (dd, 1H), 4.48 (m, 1H), 3.90 (m, 2H), 3.74 (m, 1H), 2.32 (m, 1H), 2.22 (m, 1H), 1.89 (s, 3H), 0.88 (s, 9H), 0.07 (s, 6H); FAB MS *m*/*z* 357 (M + H$^+$).

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl N-Methyl-N-(2-bromoethyl)phosphoramidic Chloride (6a). Triethylamine (12.24 g, 121 mmol) was added dropwise to a stirred solution of **4a**[11] (12.04 g, 55 mmol) and phosphorus oxychloride (8.44 g, 55-mmol) in CH_2Cl_2 (100 mL) at 0 °C under N_2. The mixture was allowed to stir overnight and then poured over ice. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were combined and dried ($MgSO_4$). The filtrate was concentrated under reduced pressure and the residue purified by chromatography (1:4 EtOAc:hexanes) to give *N*-methyl-*N*-(2-bromoethyl)phosphoramidic dichloride as an oil (13.00 g, 93%): R_f 0.67 (1:4 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 3.65 (dt, 2H, $J_{H,P}$ = 14.1Hz), 3.50 (t, 2H), 2.93 (d, 3H, $J_{H,P}$ = 15.5 Hz); ^{31}P NMR ($CDCl_3$) δ −6.57.

Butyllithium (10.8 mL, 21.60 mmol, 2.0 M) was added dropwise at 0 °C to a stirred solution of **5** (2.87 g, 19.60 mmol) and a crystal of 4-(phenylazo)diphenylamine indicator in THF (7 mL) under N_2. The resulting solution was added dropwise at −78 °C to a stirred solution of *N*-methyl-*N*-(2-bromoethyl)-phosphoramidic dichloride (5.00 g, 19.60 mmol) in THF (10 mL) under N_2. The mixture was allowed to stir under N_2 at −78 °C for 3 h, at which time the purple mixture turned yellow. The mixture was warmed to room temperature and the solvent removed under reduced pressure. The residue was purified by chromatography (1:4 EtOAc:hexanes) and the product isolated as an oil (4.77 g, 67%): R_f 0.61 (1:1 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 4.26 (dt, 2H), 4.17 (t, 1H), 4.06 (t, 1H), 3.54 (t, 1H), 3.44 (m, 4H), 2.76 (d, 3H, $J_{H,P}$ = 12.8 Hz), 1.95 (dt, 2H), 1.70 (s, 3H), 1.64 (s, 3H); ^{31}P NMR ($CDCl_3$) δ −8.07.

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl N-methyl-N-(2-chloroethyl)phosphoramidic chloride (6b): prepared as described for **6a** using 14 mmol of **5** and adjusting the other reagents accordingly. The crude product was purified by chromatography (1:4 EtOAc:hexanes) to give **6b** as an oil (2.72 g, 61%): R_f 0.75 (1:1 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 4.22 (m, 4H), 3.60 (m, 3H), 3.42 (m, 2H), 2.80 (d, 3H), 1.99 (dt, 2H), 1.39 (s, 3H), 1.33 (s, 3H); ^{31}P NMR ($CDCl_3$) δ −7.69, −7.92 (1:1 ratio of diastereomers).

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl N-methyl-N-(2-iodoethyl)phosphoramidic choride (6c): prepared as described for **6a** using 10 mmol of **5** and adjusting the other reagents accordingly. The crude product was purified by chromatography (1:4 EtOAc:hexanes) which afforded a pale yellow oil (2.50 g, 61%): R_f 0.67 (1:1 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 4.27 (m, 3H), 4.10 (t, 1H), 3.59 (t, 1H), 3.44 (m, 2H), 3.26 (t, 2H), 2.78 (d, 3H), 2.00 (dt, 2H), 1.41 (s, 3H), 1.35 (s, 3H); ^{31}P NMR ($CDCl_3$) δ −8.46, −8.69 (1:1 ratio of diastereomers).

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl bis(2-bromoethyl)phosphoramidic chloride (6e): prepared as described for **6a** using 12 mmol of **5** and adjusting the other reagents accordingly. The crude product was purified by chromatography (1:4 EtOAc:hexanes) to give **6e** as an oil (3.55 g, 65%): R_f 0.74 (1:1 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 4.33 (m, 2H), 4.20 (m, 1H), 4.11 (dd, 1H), 3.56 (m, 9H), 2.05 (m, 2H), 1.36 (s, 3H), 1.30 (s, 3H); ^{31}P NMR ($CDCl_3$) δ −9.86. Anal. ($C_{11}H_{21}Br_2ClNO_4P$) C, H, N.

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl morpholinophosphoramidic chloride (6f): prepared as described for **6a** using 20 mmol of **5** and adjusting the other reagents accordingly. The crude product was purified by chromatography (1:4 EtOAc:hexanes) to give **6f** as a yellow oil (3.45 g, 55%): R_f 0.54 (1:1 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 4.27 (m, 2H), 4.16 (m, 1H), 4.05 (t, 1H), 3.67 (m, 4H), 3.54 (t, 1H), 3.13 (m, 4H), 1.96 (m, 2H), 1.35 (s, 3H), 1.29 (s, 3H); ^{31}P NMR ($CDCl_3$) δ −10.48, −10.85 (1:1 mixture of diastereomers).

2-(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl piperidinophosphoramidic chloride (6g): prepared as described for **6a** using 20 mmol of **5** and adjusting the other reagents accordingly. The crude product was purified by chromatography (1:4 EtOAc:hexanes) to give **6g** (2.69 g, 43%): R_f 0.65 (1:4 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 4.23 (m, 3H), 4.08 (dd, 1H), 3.56 (dd, 1H), 3.12 (m, 4H), 1.97 (dt, 2H), 1.58 (m, 6H), 1.39 (s, 3H), 1.33 (s, 3H); ^{31}P NMR ($CDCl_3$) δ −9.70, −9.87 (1:1 ratio of diastereomers); FAB MS *m*/*z* 312 (M + H)$^+$.

3′-O-(*tert*-Butyldimethylsilyl)-5-fluoro-2′-deoxy-5′-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl N-Methyl-N-(2-bromoethyl)phosphoramidate (8a). Lithium diisopropylamide was prepared by adding butyllithium (1.38 mL, 2.75 mmol, 2.0 M) to a stirred solution of diisopropylamine (0.39 mL, 2.75 mmol) in THF (1 mL) at 0 °C under N_2. The mixture was allowed to stir at 0 °C for 10 min. This solution was added dropwise to a stirred solution of **7** (0.45 g, 1.25 mmol) in THF (5 mL) at 0 °C under N_2. This alkoxide solution was then added dropwise to a stirred solution of **6a** (0.46 g, 1.25 mmol) in THF (5 mL) at 0 °C under N_2, and stirring was continued overnight at room temperature. The solvent was then removed under reduced pressure and the residue purified by chromatography (EtOAc) to give **8a** (0.72 g, 71%): R_f 0.71 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.79 (m, 1H), 6.27 (m, 1H), 4.44 (m, 1H), 4.11 (m, 7H), 3.56 (t, 1H), 3.46 (m, 4H), 2.72 (d, 3H), 2.30 (m, 1H), 2.03 (m, 1H), 1.93 (m, 2H), 1.39 (s, 3H), 1.33 (s, 3H), 0.87 (s, 9H), 0.08 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.30, −14.50 (diastereomers); LSIMS *m*/*z* 688 (M + H)$^+$.

3′-O-(*tert*-Butyldimethylsilyl)-5-fluoro-2′-deoxy-5′-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl N-methyl-

N-(2-chloroethyl)phosphoramidate (8b): prepared as described for **8a** (1.4-mmol scale). The crude residue was purified by chromatography (EtOAc) to give **8b** (725 mg, 81%): R_f 0.75 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.81 (m, 1H), 6.27 (m, 1H), 4.43 (m, 1H), 4.11 (m, 7H), 3.59 (m, 3H), 3.37 (m, 2H), 2.72 (d, 3H), 2.28 (m, 1H), 2.05 (m, 1H), 1.92 (m, 2H), 1.38 (s, 3H), 1.32 (s, 3H), 0.88 (s, 9H), 0.08 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.19, −14.44 (diastereomers); FAB MS m/z 644 $(M + H)^+$.

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl N-methyl-N-(2-iodoethyl)phosphoramidate (8c): prepared as described for **8a** (1.1-mmol scale). The crude residue was purified by chromatography (EtOAc) to give **8c** (503 mg, 62%): R_f 0.70 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.79 (m, 1H), 6.22 (dt, 1H), 4.39 (m, 1H), 4.08 (m, 7H), 3.53 (m, 1H), 3.35 (m, 2H), 3.21 (m, 2H), 2.65 (d, 3H), 2.29 (m, 1H), 2.05 (m, 1H), 1.93 (m, 2H), 1.34 (s, 3H), 1.28 (s, 3H), 0.83 (s, 9H), 0.04 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.39, −14.65 (diastereomers).

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl N-Methyl-N-(2-tosylethyl)phosphoramidate (8d). Silver tosylate (1.09 g, 3.91 mmol) was added to a solution of **8a** (0.67 g, 0.98 mmol) in CH_3CN (2.5 mL). The mixture was refluxed for 3 h. The mixture was cooled and filtered and the solvent removed under reduced pressure to afford **8d** as an oil (0.70 g, 92%): R_f 0.75 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.74 (m, 3H), 7.31 (d, 2H), 6.24 (m, 1H), 4.41 (m, 1H), 4.00 (m, 9H), 3.52 (t, 1H), 3.28 (m, 2H), 2.65 (d, 3H), 2.40 (s, 3H), 2.22 (m, 1H), 2.02 (m, 1H), 1.88 (m, 2H), 1.35 (s, 3H), 1.29 (s, 3H), 0.84 (s, 9H), 0.05 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −13.38, −12.92; FAB MS m/z 780 $(M + H)^+$.

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl bis(2-bromoethyl)phosphoramidate (8e): prepared as described for **8a** (1.54-mmol scale). The crude product was purified by chromatography (EtOAc) to give **8e** (931 mg, 81%): R_f 0.70 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.68 (m, 1H), 6.25 (m, 1H), 4.46 (m, 1H), 4.18 (m, 7H), 3.57 (m, 1H), 3.45 (m, 8H), 2.30 (m, 1H), 2.18 (m, 1H), 1.92 (m, 2H), 1.40 (s, 3H), 1.36 (s, 3H), 0.89 (s, 9H), 0.08 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −15.20, −15.35 (diastereomers).

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'deoxy-5'-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl morpholinophosphoramidate (8f): prepared as described for **8a** (1.2-mmol scale). The crude product was purified by chromatography (EtOAc) to give **8f** (492 mg, 68%): R_f 0.72 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.74 (m, 1H), 6.22 (m, 1H), 4.38 (m, 1H), 4.06 (m, 6H), 3.88 (m, 1H), 3.62 (m, 4H), 3.54 (t, 1H), 3.11 (m, 4H), 2.25 (m, 1H), 2.01 (m, 1H), 1.88 (m, 2H), 1.35 (s, 3H), 1.29 (s, 3H), 0.84 (s, 9H), 0.05 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −16.15, −16.47 (diastereomers); FAB MS m/z 638 $(M + H)^+$.

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl piperidinophosphoramidate (8g): prepared as described for **8a** (0.87-mmol scale). The crude product was purified by chromatography (EtOAc) to give **8g** (473 mg, 86%): R_f 0.73 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 1H), 6.25 (dt, 1H), 4.13 (m, 7H), 3.52 (dd, 1H), 3.32 (m, 4H), 2.30 (m, 1H), 2.02 (m, 1H), 1.92 (m, 2H), 1.55 (m, 6H), 1.35 (s, 3H), 1.29 (s, 3H), 0.83 (s, 9H), 0.03 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −15.07, −15.36 (diastereomers); FAB MS m/z 636 $(M + H)^+$.

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(4,4,6-Trimethyltetrahydro-1,3-oxazin-2-yl)ethyl] N-Methyl-N-(2-bromoethyl)phosphoramidate (9a). 8a (340 mg, 0.50 mmol) was dissolved in 80% aqueous acetic acid (2 mL), heated to 80 °C for 30 min, cooled, and neutralized with NaOH. The solution was lyophilized, the residue was taken up in THF, the salts were removed by filtration, and the residue was concentrated under reduced pressure to afford the diol as an oil (270 mg, 83%): R_f 0.54 (1:9 MeOH:CH_2Cl_2); ^{31}P NMR δ −13.53, −13.93, −14.06 (diastereomers).

To a solution of the diol (220 mg, 0.35 mmol) in THF (3 mL) was added $NaIO_4$ (80 mg, 0.39 mmol) in acetate buffer (3 mL, 1 M in H_2O, pH 5.0). The mixture was allowed to stir for 10 min, and the THF was then removed under reduced pressure. The mixture was lyophilized, the residue was taken up in THF, the salts were filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (3 mL), and 4-methyl-4-amino-2-pentanol (40 mg, 0.35 mmol) in CH_2Cl_2 (2 mL) was added. The mixture was allowed to stir over 3 Å molecular sieves for 15 min. The sieves were removed, and the solvent was removed under reduced pressure. The residue was purified by chromatography (2% MeOH:EtOAc) to afford **9a** (161 mg, 65%): R_f 0.69 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.75 (m, 1H), 6.23 (m, 1H), 4.40 (m, 2H), 4.04 (m, 5H), 3.74 (m, 1H), 3.43 (m, 4H), 2.69 (d, 3H), 2.27 (m, 1H), 2.01 (m, 1H), 1.87 (m, 2H), 1.41 (d, 1H), 1.14 (m, 10H), 0.85 (s, 9H), 0.05 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.30, −14.52 (diastereomers).

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl N-methyl-N-(2-chloroethyl)phosphoramidate (9b): prepared as described for **9a** (1.13-mmol scale). The crude residue was purified by chromatography (2% MeOH:EtOAc) to afford **9b** (217 mg, 30%): R_f 0.73 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 1H), 6.26 (m, 1H), 4.40 (m, 2H), 4.05 (m, 5H), 3.74 (m, 1H), 3.60 (m, 2H), 3.36 (m, 2H), 2.71 (d, 3H), 2.29 (m, 1H), 2.03 (m, 1H), 1.94 (m, 2H), 1.42 (d, 1H), 1.13 (m, 10H), 0.86 (s, 9H), 0.05 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.20, −14.42 (diastereomers).

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl N-methyl-N-(2-iodoethyl)phosphoramidate (9c): prepared as described for **9a** (0.68-mmol scale). The crude residue was purified by chromatography (2% MeOH:EtOAc) to afford **9c** (166 mg, 32%): R_f 0.73 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.73 (m, 1H), 6.17 (m, 1H), 4.33 (m, 2H), 4.03 (m, 5H), 3.66 (m, 1H), 3.32 (m, 2H), 3.22 (m, 2H), 2.60 (d, 3H), 2.26 (m, 1H), 2.03 (m, 1H), 1.87 (m, 2H), 1.34 (d, 1H), 1.08 (m, 10H), 0.78 (s, 9H); ^{31}P NMR ($CDCl_3$) δ −14.52, −14.77, −15.06, −15.31 (diastereomers).

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl N-methyl-N-(2-tosylethyl)phosphoramidate (9d): prepared as described for **9a** (0.90-mmol scale). The crude product was purified by chromatography (2% MeOH:EtOAc) to afford **9d** (333 mg, 46%): R_f 0.73 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.81 (m, 1H), 7.34 (d, 2H), 6.25 (m, 1H), 4.44 (m, 2H), 4.11 (m, 5H), 3.93 (m, 1H), 3.74 (m, 2H), 3.42 (m, 2H), 2.68 (d, 3H), 2.44 (s, 3H), 2.26 (m, 1H), 2.03 (m, 1H), 1.90 (m, 2H), 1.42 (m, 1H), 1.13 (m, 10H), 0.88 (s, 9H), 0.08 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.13, −14.36 (diastereomers).

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl morpholinophosphoramidate (9f): prepared as described for **9a** (0.81-mmol scale). The crude product was purified by chromatography (2% MeOH:EtOAc) to give **9f** (178 mg, 33%): R_f 0.70 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.75 (m, 1H), 6.23 (m, 1H), 4.38 (m, 2H), 4.07 (m, 5H), 3.71 (m, 1H), 3.63 (m, 4H), 3.12 (m, 4H), 2.27 (m, 1H), 2.02 (m, 1H), 1.89 (m, 2H), 1.41 (d, 1H), 1.07 (m, 10H), 0.86 (s, 9H), 0.06 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −16.08, −16.39 (diastereomers).

3'-O-(tert-Butyldimethylsilyl)-5-fluoro-2'-deoxy-5'-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl piperidinophosphoramidate (9g): prepared as described for **9a** (0.62-mmol scale). The crude product was purified by chromatography (2% MeOH:EtOAc) to afford **9g** (67 mg, 17%): R_f 0.70 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 1H), 6.29 (m, 1H), 4.45 (m, 1H), 4.39 (m, 1H), 4.09 (m, 5H), 3.71 (m, 1H), 3.08 (m, 4H), 2.31 (m, 1H), 2.09 (m, 1H), 1.90 (m, 2H), 1.53 (m, 6H), 1.42 (d, 1H), 1.12 (m, 10H), 0.89 (s, 9H), 0.08 (s, 6H); ^{31}P NMR ($CDCl_3$) δ -15.03, −15.25 (diastereomers).

5-Fluoro-2'-deoxy-5'-uridyl 2-(4,4,6-Trimethyltetrahydro-1,3-oxazin-2-yl)ethyl] N-methyl-N-(2-bromoethyl)-phosphoramidate (1a). Tetrabutylammonium fluoride (0.15 mL, 0.15 mmol, 1.0 M in THF) was added at 0 °C to a stirred solution of **9a** (0.105 g, 0.15 mmol) in THF (3 mL). The mixture was allowed to stir for 20 min at 0 °C. The solvent was removed under reduced pressure and the residue purified by chromatography (3% MeOH:EtOAc) to afford **1a** (33 mg, 37%): R_f 0.40 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.79 (m,

1H), 6.22 (m, 1H), 4.51 (m, 1H), 4.45 (m, 1H), 4.25 (m, 5H), 3.74 (m, 1H), 3.48 (m, 4H), 2.72 (d, 3H), 2.45 (m, 1H), 2.16 (m, 1H), 1.92 (m, 2H), 1.43 (d, 1H), 1.10 (m, 9H), 0.91 (d, 1H); ^{31}P NMR ($CDCl_3$) δ −13.29, −13.65, −13.69, −13.81 (diastereomers); FAB MS ($C_{21}H_{36}N_4O_8PFBr$) calcd 601.1440, obsd 601.1445.

5-Fluoro-2′-deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-chloroethyl)phosphoramidate (1b): prepared as described for **1a** (0.32-mmol scale). The crude product was purified by chromatography (3% MeOH:EtOAc) to afford **1b** (51 mg, 28%): R_f 0.43 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.76 (m, 1H), 6.21 (m, 1H), 4.51 (m, 1H), 4.40 (m, 1H), 4.19 (m, 5H), 3.76 (m, 1H), 3.60 (m, 2H), 3.33 (m, 2H), 2.74 (d, 3H), 2.45 (m, 1H), 2.17 (m, 1H), 1.91 (m, 2H), 1.43 (d, 1H), 1.06 (m, 9H), 0.91 (d, 1H); ^{31}P NMR ($CDCl_3$) δ −13.60, −13.78 (diastereomers); FAB MS ($C_{21}H_{36}N_4O_8PClF$) calcd 557.1946, obsd 557.1962.

5-Fluoro-2′-deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-iodoethyl)phosphoramidate (1c): prepared as described for **1a** (0.22-mmol). The crude product was purified by chromatography (3% MeOH:EtOAc) to afford **1c** (40 mg, 28%): R_f 0.34 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.74 (m, 1H), 6.18 (m, 1H), 4.48 (m, 1H), 4.45 (m, 1H), 4.19 (m, 5H), 3.72 (m, 1H), 3.34 (m, 2H), 3.26 (m, 2H), 2.65 (d, 3H), 2.40 (m, 1H), 2.14 (m, 1H), 1.94 (m, 2H), 1.44 (d, 1H), 1.12 (m, 10H); ^{31}P NMR ($CDCl_3$) δ −13.75, −14.04 (diastereomers); FAB MS ($C_{21}H_{36}N_4O_8PFI$) calcd 649.1302, obsd 649.1315.

5-Fluoro-2′-deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-tosylethyl)phosphoramidate (1d): prepared as described for **1a** (0.41-mmol scale). The crude product was purified by chromatography (3% MeOH:EtOAc) to give **1d** (37 mg, 13%): R_f 0.42 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.76 (m, 3H), 7.36 (d, 2H), 6.20 (m, 1H), 4.48 (m, 2H), 4.17 (m, 6H), 3.76 (m, 2H), 3.30 (m, 2H), 2.69 (d, 3H), 2.45 (s, 3H), 2.22 (m, 1H), 2.05 (m, 1H), 1.94 (m, 2H), 1.40 (d, 1H), 1.15 (m, 9H), 0.10 (d, 1H); ^{31}P NMR ($CDCl_3$) δ −13.30, −13.58 (diastereomers); FAB MS ($C_{28}H_{42}N_4O_{11}PSF$) calcd 693.2373, obsd 693.2385.

5-Fluoro-2′-deoxy-5′-uridyl 2-(4,4,6-Trimethyltetrahydro-1,3-oxazin-2-yl)ethyl] Bis(2-Bromoethyl)phosphoramidate (1e). A solution of **8e** (931 mg, 1.25-mmol) in 80% aqueous acetic acid (5 mL) was heated to 80 °C for 30 min, cooled, and neutralized with NaOH. The solution was lyophilized, the residue was taken up in THF, the salts were removed by filtration, and the solution was concentrated under reduced pressure. The crude residue was purified by chromatography (3% MeOH:EtOAc) to give the diol (528 mg, 60%): R_f 0.60 (1:9 MeOH:CH_2Cl_2).

Aqueous HF (0.25 mL, 50% in H_2O) was added to a stirred solution of the diol (255 mg, 0.36-mmol) in CH_3CN (4.8 mL). The mixture was allowed to stir for 1 h and then neutralized with NaOH. The solution was lyophilized, the residue was taken up in THF, the salts were removed by filtration, and the filtrate was concentrated under reduced pressure to afford the triol (163 mg, 73%): R_f 0.36 (1:9 MeOH:CH_2Cl_2).

To a solution of the triol (163 mg, 0.26-mmol) in THF (3 mL) was added sodium periodate in aqueous acetate buffer (3 mL, pH 5.0, 1 M). The mixture was allowed to stir for 7 min, and the THF was then removed under reduced pressure. The solution was lyophilized, the residue was taken up in THF, the salts were filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (5 mL), and the amino alcohol (31 mg, 0.26-mmol) in CH_2Cl_2 (3 mL) was added. The mixture was allowed to stir over 3 Å molecular sieves for 15 min. The sieves were removed, and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography (3% MeOH:EtOAc) to give **1e** (17 mg, 10%): R_f 0.47 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.69 (m, 1H), 6.20 (m, 1H), 4.48 (m, 1H), 4.42 (m, 1H), 4.15 (m, 5H), 3.78 (m, 1H), 3.44 (m, 8H), 2.43 (m, 1H), 2.16 (m, 1H), 2.03 (s, 3H), 1.95 (m, 2H), 1.44 (d, 1H), 1.15 (m, 10H); ^{31}P NMR ($CDCl_3$) δ −14.73, −14.85 (diastereomers); FAB MS ($C_{22}H_{36}N_4O_8PFBr_2$) calcd 693.0701, obsd 693.0705.

5-Fluoro-2′-deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl morpholinophosphoramidate (1f): prepared as described for **1a** (0.27-mmol scale). The crude residue was purified by chromatography (3% MeOH:EtOAc) to give **1e** (65 mg, 44%): R_f 0.36 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.67 (m, 1H), 6.15 (m, 1H), 4.40 (m, 2H), 4.08 (m, 5H), 3.70 (m, 1H), 3.59 (m, 4H), 3.08 (m, 4H), 2.36 (m, 1H), 2.02 (m, 1H), 1.88 (m, 2H), 1.39 (d, 1H), 1.16 (m, 10H); ^{31}P NMR ($CDCl_3$) δ −15.42, −15.71, −15.83 (diastereomers). Anal. ($C_{22}H_{36}N_4O_8PF$) C, H, N.

5-Fluoro-2′-deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl piperidinophosphoramidate (1g): prepared as described for **1a** (0.10-mmol scale). The crude product was purified by chromatography (3% MeOH:EtOAc) to afford **1g** (10 mg, 18%): R_f 0.70 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.75 (m, 1H), 6.21 (m, 1H), 4.52 (m, 1H), 4.42 (m, 1H), 4.12 (m, 5H), 3.71 (m, 1H), 3.09 (m, 4H), 2.42 (m, 1H), 2.19 (m, 1H), 1.91 (m, 2H), 1.52 (m, 6H), 1.41 (d, 1H), 1.13 (m, 10H); ^{31}P NMR ($CDCl_3$) δ −14.13, −14.36 (diastereomers); FAB MS ($C_{23}H_{39}N_4O_8PF$) calcd 549.2492, obsd 549.2498.

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl *N*-methyl-*N*-(2-bromoethyl)phosphoramidate (11a): prepared as described for **8a** using **10** on a 1.46-mmol scale. The crude product was purified by chromatography (EtOAc) to give **11a** (718 mg, 73%): R_f 0.75 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.60 (dd, 1H), 6.21 (dt, 1H), 5.68 (d, 1H), 4.36 (m, 1H), 4.07 (m, 7H), 3.52 (m, 1H), 3.39 (m, 4H), 2.65 (d, 3H), 2.25 (m, 1H), 2.02 (m, 1H), 1.86 (m, 2H), 1.33 (s, 3H), 1.27 (s, 3H), 0.82 (s, 9H), 0.02 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.30, 14.56 (diastereomers). Anal. ($C_{25}H_{45}N_3O_9PBrSi$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-uridyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl *N*-methyl-*N*-(2-chloroethyl)phosphoramidate (11b): prepared as described for **8a** using **10** on a 1.59-mmol scale. The residue was purified by chromatography (EtOAc) to afford **11b** (0.69 g, 69%) as an oil: R_f 0.75 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.70 (m, 1H), 6.26 (dt, 1H), 5.73 (d, 1H), 4.22 (m, 1H), 4.10 (m, 7H), 3.60 (m, 3H), 3.40 (m, 2H), 2.75 (d, 3H), 2.30 (m, 1H), 2.07 (m, 1H), 1.92 (m, 2H), 1.40 (s, 3H), 1.36 (s, 3H), 0.84 (s, 9H), 0.08 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.54, −14.74 (diastereomers).

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-uridyl 2(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl bis(2−bromoethyl)phosphoramidate (11e): prepared as described for **8a** using **10** on a 1.46-mmol scale. The residue was purified by chromatography (EtOAc) to give **11e** (765 mg, 68%): R_f 0.75 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.52 (m, 1H), 6.19 (dt, 1H), 5.72 (d, 1H), 4.35 (m, 1H), 4.07 (m, 7H), 3.52 (dd, 1H), 3.42 (m, 8H), 2.24 (m, 1H), 2.08 (m, 1H), 1.89 (m, 2H), 1.35 (s, 3H), 1.29 (s, 3H), 0.89 (s, 9H), 0.03 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −15.76, −15.88 (diastereomers).

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-bromoethyl)phosphoramidate (12a): prepared as described for **9a** using **11a** (0.7-mmol scale). The crude product was purified by chromatography (2% MeOH:EtOAc) to give **12a** (203 mg, 42%): R_f = 0.71 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) 7.61 (d, 1H), 6.24 (m, 1H), 5.70 (d, 1H), 4.36 (m, 2H), 4.09 (m, 5H), 3.71 (m, 1H), 3.41 (m, 4H), 2.68 (d, 3H), 2.29 (m, 1H), 2.03 (m, 1H), 1.85 (m, 2H), 1.41 (d, 1H), 1.09 (m, 10H), 0.85 (s, 9H), 0.05 (s, 6H); ^{31}P NMR ($CDCl_3$) −14.35, −14.72 (diastereomers).

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-chloroethyl)phosphoramidate (12b): prepared as described for **9a** using **11b** (0.87-mmol scale). The crude product was purified by chromatography (2% MeOH:EtOAc) to afford **12b** (101 mg, 17%): R_f 0.74 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.65 (m, 1H), 6.25 (m, 1H), 5.73 (d, 1H), 4.42 (m, 2H), 4.07 (m, 5H), 3.75 (m, 1H), 3.62 (m, 2H), 3.37 (m, 2H), 2.72 (d, 3H), 2.27 (m, 1H), 2.05 (m, 1H), 1.95 (m, 2H), 1.42 (d, 6H), 1.10 (m, 10H), 0.84 (s, 9H), 0.05 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.56, −14.83 (diastereomers).

2′-Deoxy-5′-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2−bromoethyl)phosphoramidate (2a): prepared as described for **1a** using **12a** (0.28-mmol scale). The crude product was purified by chromatography (3% MeOH:EtOAc) to give **2a** (24 mg, 15%): R_f 0.38

(1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.66 (m, 1H), 6.20 (m, 1H), 5.74 (d, 1H), 4.56 (m, 1H), 4.41 (m, 1H), 4.13 (m, 5H), 3.56 (m, 1H), 3.43 (m, 4H), 2.72 (d, 3H), 2.37 (m, 1H), 2.07 (m, 1H), 1.81 (m, 2H), 1.79 (d, 1H), 1.09 (m, 10H); ^{31}P NMR ($CDCl_3$) δ −13.71, −13.88 (diastereomers); FAB MS ($C_{21}H_{37}N_4O_8PBr$) calcd 583.1534, obsd 583.1551.

2'-Deoxy-5'-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-chloroethyl)phosphoramidate (2b): prepared as described for **1a** using **12b** (0.15-mmol scale). The crude product was purified by chromatography (3% MeOH:EtOAc) to afford **2b** (24 mg, 24%): R_f 0.45 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.6 (m, 1H), 6.21 (m, 1H), 5.73 (d, 1H), 4.50 (m, 1H), 4.41 (m, 1H), 4.20 (m, 5H), 3.77 (m, 1H), 3.61 (m, 2H), 3.30 (m, 2H), 2.76 (d, 3H), 2.45 (m, 1H), 2.19 (m, 1H), 1.94 (m, 2H), 1.42 (d, 1H), 1.06 (m, 9H), 0.91 (d, 1H); ^{31}P NMR ($CDCl_3$) δ −13.89, −14.03 (diastereomers). FAB MS ($C_{21}H_{37}N_4O_8PCl$): calcd 539.2040; obsd 539.2046.

2'-Deoxy-5'-uridyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl bis(2-bromoethyl)phosphoramidate (2e): prepared as described for **1e** using **11e** (0.53-mmol scale). The crude product was purified by chromatography (3% MeOH:EtOAc) to give **2e** (120 mg, 33%): R_f 0.53 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.52 (m, 1H), 6.22 (m, 1H), 5.74 (d, 1H), 4.44 (m, 1H), 4.36 (m, 1H), 4.12 (m, 5H), 3.74 (m, 1H), 3.42 (m, 8H), 2.43 (m, 1H), 2.13 (m, 1H), 1.41 (d, 1H), 1.11 (m, 9H), 1.01 (d, 1H); ^{31}P NMR ($CDCl_3$) δ −15.85, −15.76 (diastereomers). Anal. ($C_{22}H_{37}N_4O_8PBr_2 \cdot H_2O$) C, H, N.

3'-*O*-(*tert*-Butyldimethylsilyl)-5'-thymidyl 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethyl *N*-methyl-*N*-(2-bromoethyl)phosphoramidate (14a): prepared as described for **8a** using **13** on a 5.48-mmol scale. The crude product was purified by chromatography (EtOAc) to give **14a** (1.75 g, 51%): R_f 0.75 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.41 (d, 1H), 6.31 (dt, 1H), 4.45 (m, 1H), 4.11 (m, 7H), 3.59 (m, 1H), 3.50 (m, 4H), 2.73 (d, 3H), 2.28 (m, 1H), 2.04 (m, 1H), 1.95 (s, 3H), 1.63 (m, 2H), 1.40 (s, 3H), 1.34 (s, 3H), 0.90 (s, 9H), 0.09 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.76, −14.58 (diastereomers). Anal. ($C_{26}H_{47}N_3O_9PBrSi$) C, H, N.

3'-*O*-(*tert*-Butyldimethylsilyl)-5'-thymidyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-bromoethyl)phosphoramidate (15a): prepared as described for **9a** using **14a** on a 2.52-mmol scale. The intermediate diol was obtained as an oil (1.00 g, 62%): R_f 0.50 (1:9 MeOH:CH_2Cl_2); ^{31}P NMR δ −13.97, −14.10. The diol was converted to **15a** on a 1.30-mmol scale and the residue purified by chromatography (2% MeOH:EtOAc) (364 mg, 48%): R_f 0.72 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) 7.42 (m, 1H), 6.30 (m, 1H), 4.40 (m, 2H), 4.09 (m, 5H), 3.79 (m, 1H), 3.42 (m, 4H), 2.72 (d, 3H), 2.31 (m, 1H), 2.05 (m, 1H), 1.91 (m, 5H), 1.43 (d, 1H), 1.15 (m, 9H), 1.01 (d, 1H), 0.89 (s, 9H), 0.09 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −14.57, −14.72 (diastereomers).

5'-Thymidyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*-methyl-*N*-(2-bromoethyl)phosphoramidate (3a): prepared as described for **1a** using **15a** on a 0.48-mmol scale. The crude product was purified by chromatography (3% MeOH:EtOAc) to give **3a** (76 mg, 26% yield): R_f 0.42 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.39 (m, 1H), 6.25 (m, 1H), 4.53 (m, 1H), 4.39 (m, 1H), 4.15 (m, 5H), 3.77 (m, 1H), 3.43 (m, 4H), 2.73 (d, 3H), 2.41 (m, 1H), 2.17 (m, 1H), 1.98 (m, 5H), 1.44 (d, 1H), 1.13 (m, 9H), 1.08 (d, 1H); ^{31}P NMR ($CDCl_3$) δ −13.78, −14.00 (diastereomers); FAB MS ($C_{22}H_{39}N_4O_8PBr$) calcd 597.1691, obsd 597.1694.

3'-*O*-(*tert*-Butyldimethylsilyl)thymidyl 2(2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl bis(2-bromoethyl)phosphoramidate (14e): prepared as described for **8e** using **13** on a 1.82-mmol scale. The crude product was purified by chromatography (EtOAc) to give **14e** (972 mg, 69%): R_f 0.71 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.34 (m, 1H), 6.24 (dt, 1H), 4.36 (m, 1H), 4.06 (m, 7H), 3.50 (m, 1H), 3.40 (m, 8H), 2.18 (m, 1H), 2.07 (m, 1H), 1.85 (m, 5H), 1.31 (s, 3H), 1.25 (s, 3H), 0.82 (s, 9H), 0.02 (s, 6H); ^{31}P NMR ($CDCl_3$) δ −16.00, −16.30 (diastereomers); FAB MS m/z 776 $(M + H)^+$.

5'-Thymidyl 2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl bis(2-bromoethyl)phosphoramidate (3e): prepared as described for **1e** using **14e** on a 0.90 mmol-scale to afford the triol (393 mg, 70%): R_f 0.33 (1:9 MeOH:CH_2Cl_2). The triol was converted to **3e** on a 0.63-mmol scale and the crude product purified by chromatography (3% MeOH:EtOAc) to give the product (167 mg, 48%): R_f 0.50 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.38 (d, 1H), 6.23 (m, 1H), 4.50 (m, 1H), 4.43 (m, 1H), 4.19 (m, 4H), 4.06 (m, 1H), 3.79 (m, 1H), 3.44 (m, 8H), 2.64 (m, 1H), 2.28 (m, 1H), 1.93 (m, 5H), 1.45 (d, 1H), 1.15 (m, 10H); ^{31}P NMR ($CDCl_3$) δ −14.95, −15.10 (diastereomers). Anal. ($C_{23}H_{39}N_4O_8PBr_2$) C, H, N.

In Vitro Cytotoxicity. Stock solutions of drugs were prepared in 95% ethanol, and serial dilutions of drugs were prepared in ethanol such that 50 μL of drug solution added to 10 mL of cell suspension gave the desired final concentration. L1210 cells in exponential growth were suspended in Fischer's medium supplemented with 10% horse serum, 1% glutamine, and 1% antibiotic−antimycotic solution to give 10-mL volumes of cell suspension at a final density of (3−6) × 10^4/mL. Appropriate volumes of the drug solution were transferred to the cell suspensions, and incubation was continued for 2, 8, 24, or 48 h. The cells were spun down, resuspended in fresh drug-free medium, and returned to the incubator. In one set of experiments (Figure 1), cell counts were determined with a coulter counter two times a day for 10 days or until plateau phase was reached. The results are reported in units of cell density. In the other set of experiments (Figure 2), cell counts were determined 48 h after drug treatment. The results are reported as percent of control values. The control cell densities were in the range (5−7) × 10^5/mL.

Clonogenic survival of B16 melanoma cells was determined according to the method of Miribelli et al.[14] B16 cells in exponential growth (2−3) × 10^6 cells in 10 mL of serum-free MEM medium) were treated with drug for 2 h. The cells were separated, washed, and resuspended in MEM medium supplemented with 10% fetal bovine serum. The cells were plated in 60-mm culture dishes at a density of 50−50 000 cells/plate (depending upon the drug concentration used) and then incubated for 8 d in a CO_2 incubator at 37 °C. The colonies were fixed and stained with 0.5% crystal violet in ethanol and counted. The log of the surviving fraction was plotted against drug concentration, and the concentration required to reduce colony formation by 2 logs (LC_{99}) was determined from the log linear portion of the curve.

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